Synthesis of Semliki-Forest virus in polyamine-depleted baby-hamster kidney cells

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The role of polyamines in macromolecular synthesis has been studied using the synthesis of Semliki-Forest virus (SF virus) in normal and α -difluoromethylornithine-treated baby-hamster kidney (BHK21) cells as a model system. The activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, the rate-limiting enzymes in polyamine biosynthesis, decreased rapidly in mock- and SF-virus-infected cells, indicating that virus production in BHK21 cells was not dependent on polyamines formed after infection. A prolonged treatment of BHK21 cells with α -difluoromethylornithine, a specific inhibitor of polyamine synthesis, resulted in a marked inhibition of the initial rate of virus production, which appeared 72 h after the beginning of the treatment. This inhibition was reversed by putrescine, spermidine and spermine, and at least partially by several other diamines and polyamine homologues. Polyamine-depletion also markedly reduced viral RNA polymerase activity in SF-virus-infected cells. Addition of spermidine to the culture medium rapidly increased viral RNA polymerase activity in the inhibitor-treated cells but had no effect on the enzyme activity when added directly to the assay mixture. The results indicated that polyamines are needed for maximum initial rate of SF-virus replication and suggest that the inhibition of virus production in polyamine-depleted cells is at least partly due to malfunction of the protein-synthetic machinery of the host cell.

Several viruses that replicate in bacterial, plant or animal cells have been reported to contain significant amounts of polyamines (for references, see Cohen & McCormick, 1979; Raina *et al.*, 1981a), but the significance of these basic compounds for viral functions and virus multiplication is still poorly understood. It has been reported that virus production in polyamine-depleted micro-organisms is markedly inhibited or delayed (Cohen & McCormick, 1979; Hafner *et al.*, 1979), whereas in animal systems, results obtained with inhibitors of polyamine synthesis have been conflicting. Methylglyoxal bis(guanyldrazone), an inhibitor of *S*-adenosylmethionine decarboxylase, reduced the yield of vaccinia and human cytomegaloviruses, whereas the synthesis of herpes simplex viruses (HSV-1 and HSV-2) was uninfluenced by this drug (McCormick & Newton, 1975; Tyms *et al.*, 1979; Tyms & Williamson, 1980) and also by DL- α -methylornithine, an inhibitor of ornithine decarboxylase (Tyms *et al.*, 1979). In one study (Tyms *et al.*, 1979) the production of human cytomegalovirus was reduced by α -methylornithine, whereas another

study (Isom & Pegg, 1979) reported no effect on the replication of the same virus by this compound or its derivative, DL- α -difluoromethylornithine. We reported recently (Tuomi *et al.*, 1980a; Raina *et al.*, 1981b) that pretreatment of BHK21 cells with α -difluoromethylornithine markedly decreased the yield of two types of viruses, a DNA virus replicating in the nucleus (HSV-2) and an RNA virus replicating in the cytoplasm (Semliki-Forest virus, SF virus). The inhibition developed remarkably slowly, i.e. a pretreatment of about 3 days was required to demonstrate the reduction of the virus yield. It seemed therefore that factors such as the duration of pretreatment with the inhibitor, the cell density at the start of the experiment and non-specific effects of the inhibitor could at least partly explain the earlier discrepant results (Tuomi *et al.*, 1980a,b; Raina *et al.*, 1981b).

This report extends our previous results on the replication of SF virus in polyamine-deficient BHK21 cells. We further demonstrate that polyamine-depletion markedly reduced the activity of viral RNA polymerase in SF-virus-infected cells, an

effect that was rapidly reversed by the addition of spermidine to the culture medium.

Experimental

Chemicals

Putrescine (1,4-diaminobutane), cadaverine (1,5-diaminopentane), spermidine and spermine, as their hydrochloride salts, were obtained from Calbiochem, Los Angeles, CA, U.S.A., and purified by chromatography and/or by recrystallization as previously described (Pajula *et al.*, 1979). 1,3-Diaminopropane (Puriss.) was purchased from Fluka A.G., Buchs SG, Switzerland, and purified by recrystallization. 1,6-Diaminohexane was a product of BDH Chemicals, Poole, Dorset, U.K. Diethylenetriamine (2,2'-diaminodiethylamine) and triethylenetetra-amine [*NN'*-bis-(2-aminoethyl)ethane-1,2-diamine] were kindly provided by Ciba-Geigy, Basle, Switzerland. *sym*-Norspermidine (3,3'-diaminodipropylamine) and *sym*-norspermine [*NN'*-bis-(3-aminopropyl)propane-1,3-diamine] were purchased from Eastman Organic Chemicals, Rochester, NY, U.S.A. Purity of these compounds was checked by paper electrophoresis (Raina, 1963).

L-[1-¹⁴C]Methionine (sp. radioactivity 60 Ci/mol), DL-[1-¹⁴C]ornithine (sp. radioactivity 59 Ci/mol) and [U-³H]UTP (sp. radioactivity 2000 Ci/mol) were products of The Radiochemical Centre, Amersham, Bucks., U.K. Labelled *S*-adenosyl-L-methionine was synthesized as described by Pegg & Williams-Ashman (1969). ATP, GTP and CTP were products of Boehringer, Mannheim, Germany. DL- α -Difluoromethylornithine was a gift from Centre de Recherche Merrell International, Strasbourg, France. Actinomycin D was a product of Merck, Sharp and Dohme, Rahway, NJ, U.S.A.

Cell cultures

Stock cultures of BHK21, a continuous cell line derived from baby hamster kidney, were grown in Eagle's minimal essential medium (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% foetal-calf serum, penicillin (200 units/ml) and streptomycin (100 μ g/ml), and passaged by trypsin treatment (Tuomi *et al.*, 1980b). A prototype strain of SF virus was obtained from Dr. L. Kääriäinen (Department of Virology, University of Helsinki, Helsinki, Finland). Determination of the growth of virus stock and of infectivity, as plaque-forming units (PFU), was performed as previously described (Tuomi *et al.*, 1980a). For determination of virus titres, on average, 80 plaques were counted in each of the duplicate dishes at an appropriate virus dilution, giving a standard deviation of 8%.

The experiments were started from confluent cultures of BHK21 cells by subculturing the cells in plastic Petri dishes (diameter 50 mm unless other-

wise indicated) at a ratio of about 1:10 (3.5×10^5 cells/dish). The cells were grown in the absence or presence of 2 mM- α -difluoromethylornithine before infection with SF virus (50 PFU/cell) (Tuomi *et al.*, 1980a,b). Where indicated, 10 μ M-polyamine and 1 mM-iproniazid (see Bethell & Pegg, 1979) were added to the cultures. After adsorption for 1 h, the maintenance medium (Eagle's minimal essential medium, containing 0.2% bovine serum albumin and the same additions as the corresponding pre-infection medium) was added. The production of SF virus was determined from samples of the medium 8 h after infection unless otherwise indicated. Virus titres were measured as PFU/ μ g of cellular DNA. If not otherwise indicated, DNA was determined at the time of infection from similarly treated but non-infected cultures. All values shown are means of at least two parallel cultures.

Enzyme assays

Assay of ornithine decarboxylase and S-adenosyl-methionine decarboxylase activities. BHK21 cells were grown for 72 h and infected with SF virus (50 PFU/cell) as described above. At the times indicated virus growth medium was removed, cells were washed twice with 0.9% NaCl, detached by scraping with a rubber policeman and collected by centrifugation. Cells were disrupted by sonication in 2 ml of 0.25 M-sucrose solution containing 0.1 mM-EDTA, 0.1 mM-pyridoxal 5'-phosphate and 1 mM-dithiothreitol. The activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase were determined from the 105 000 g_{av} supernatant fraction of the sonicated preparation (Eloranta *et al.*, 1976).

Viral RNA polymerase. The isolation of RNA replication complex from SF-virus-infected cells and the assay of viral RNA polymerase activity were carried out as described by Ranki & Kääriäinen (1979). BHK21 cells were grown in the presence or absence of 2 mM- α -difluoromethylornithine. At appropriate times the culture medium was removed and virus inoculum was added to the cells in Eagle's minimal essential medium containing 2 μ g of actinomycin D/ml. After adsorption for 1 h, cells were washed and minimal medium supplemented with 0.2% bovine serum albumin, 2 μ g of actinomycin D/ml, and, where indicated, 10 μ M-spermidine and 1 mM-iproniazid, was added. At indicated times after infection the medium was removed by suction and the dishes were immediately placed on an ice-cold steel plate. Cells were then washed with ice-cold 0.9% NaCl and collected by centrifugation. After hypo-osmotic treatment of cells for 15 min at 0°C in 10 mM-Tris/HCl, pH 8.0, containing 10 mM-NaCl, cells were broken in a Dounce homogenizer (B. Braun Melsungen A.G., Melsungen, Germany) by 15 strokes. Nuclei were pelleted at 250 g for

5 min. The supernatant was centrifuged at 15000 *g* for 20 min to obtain the mitochondrial pellet, which was used as the source of RNA polymerase. The hypo-osmotic buffer and centrifuge tubes had been boiled in the presence of 0.02% diethyl pyrocarbonate (Sigma Chemical Co., St. Louis, MO, U.S.A.) to inactivate ribonuclease (see Palmiter, 1974).

The standard reaction mixture (total volume 0.25–0.4 ml) for the assay of viral RNA polymerase activity contained 100 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, adjusted to pH 8.0 with KOH solution, 10 mM-MgCl₂, 6 mM-2-mercaptoethanol, 10 mM-phosphoenolpyruvate (Sigma), 25 µg of pyruvate kinase/ml (Sigma), 5–10 µg of actinomycin D/ml, 0.05 mM each of ATP, GTP and CTP, and 0.05 mM-[³H]UTP. After incubation for 15 min at 37°C, the reaction was stopped with 2 ml of 10% trichloroacetic acid after addition of 200 µg of bovine serum albumin. The tubes were allowed to stand on ice for 20 min, the precipitates were spun down and the supernatants discarded. The precipitates were washed three times with 10% trichloroacetic acid (see Ranki & Kääriäinen, 1970) and finally dissolved in 0.3 ml of 0.1 M-NaOH and analysed for radioactivity in Aquasol (New England Nuclear Corp., Boston, MA, U.S.A.).

Analytical methods

DNA was determined from a hot HClO₄ extract as described by Giles & Myers (1965).

Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Polyamine synthesis in SF-virus-infected cells

We have previously reported that pretreatment of BHK21 cells in culture with α-difluoromethylorni-

thine for 3 days markedly reduced the production of HSV-2 and SF virus (Tuomi *et al.*, 1980a; Raina *et al.*, 1981b). Our result with HSV-2 was contradictory to previous reports by McCormick & Newton (1975) and Tyms *et al.* (1979), who observed no effect on the replication of herpes viruses with inhibitors of polyamine synthesis. Furthermore it did not support the suggestion by Tyms and co-workers (Tyms *et al.*, 1979; Tyms & Williamson, 1980) that stimulation of cellular polyamine synthesis after virus infection (as seen in human cytomegalovirus-infected cells, but not after HSV-2 infection) would be indicative of whether an inhibitor of polyamine synthesis would be effective or not. As shown in Table 1, both in cells infected with SFV and in sham-infected (control) cells the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase, the rate-limiting enzymes in polyamine biosynthesis (Raina & Jänne, 1975; Jänne *et al.*, 1978), rapidly declined, probably due to the shift of the cultures from a serum-containing to a serum-free medium. Similar results were obtained with HSV-2-infected cultures (Raina *et al.*, 1981a). We conclude that the production of SF virus and HSV-2 in BHK21 cells is not dependent on polyamines produced after infection. Isom & Pegg (1979) reached a similar conclusion in the case of human cytomegalovirus.

Kinetics and reversal of the inhibition of SF virus production by α-difluoromethylornithine

In agreement with our previous observations (Tuomi *et al.*, 1980a), Fig. 1 demonstrates that inhibition of SF-virus production by α-difluoromethylornithine appeared slowly, i.e. only after pretreatment of cells for 3 days with the drug. Treatment of BHK21 cells for 4 days with α-difluoromethylornithine decreased the virus yield to less than 1% (Fig. 1). At each time the inhibition was fully reversed by 10 µM-spermidine.

Table 1. Effect of SF virus infection on the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase in BHK21 cells

BHK21 cells were seeded on plastic Petri dishes (diameter 90 mm; 9×10^5 cells/dish) and grown for 72 h in Eagle's minimal essential medium supplemented with 10% foetal-calf serum before infection with SF virus. The medium used for virus growth contained 0.2% bovine serum albumin but no serum. At the times indicated the cells were harvested and treated as described in the Experimental section. The values are means of duplicate assays of two cultures. N.D., not determined.

Time after infection (h)	Ornithine decarboxylase (pmol of CO ₂ /30 min per mg of protein)		S-Adenosylmethionine decarboxylase (pmol of CO ₂ / 30 min per mg of protein)	
	Control	SF virus	Control	SF virus
0	22.2	N.D.	164	N.D.
1	6.7	7.8	136	170
2	4.4	3.9	116	103
4	2.9	2.0	68	35
8	0.1	0.3	22	4

In cell cultures infected with SF virus at 37°C, the virus titre of the medium begins to increase 3–4 h after infection and the one-step growth cycle is usually completed after 6–10 h (Kääriäinen &

Fig. 1. Reversal by spermidine of the inhibition of virus production in BHK21 cells after a prolonged treatment with α -difluoromethylornithine

BHK21 cells (2×10^5 cells/dish) were grown for 2–4 days in the presence or absence of 2 mM- α -difluoromethylornithine. Iproniazid (1 mM) was added to all cultures and spermidine ($10 \mu\text{M}$) to the cultures shown 24 h before infection with SF virus (50 PFU/cell). Virus titre was determined 8 h after infection. Each point represents the mean of duplicate assays of two separate cultures. ○, Control cultures; △, α -difluoromethylornithine; ▲, α -difluoromethylornithine plus spermidine.

Söderlund, 1978). As shown in Table 2, the virus titre of the control cultures increased rapidly for 8–12 h after infection and then levelled off. The virus yield in α -difluoromethylornithine-treated cultures was markedly reduced for 8 h and thereafter gradually approached the control values. The relatively weak inhibition of virus production by α -difluoromethylornithine can be attributed to the unusually high initial cell density in these particular experiments. The results presented in Table 2 indicate that polyamine-depleted cells produced virus at a lower rate but that the increase in the virus titre continued longer as compared with the untreated cultures. On the basis of the data in Table 3 it appears unlikely that the slow production of viruses in α -difluoromethylornithine-treated cultures would primarily be due to inefficient adsorption, because virus yield of the inhibitor-treated cells infected with 50 PFU/cell was only 6% that of the control cultures infected with 5 PFU/cell (the virus titres were 1.1×10^6 and 1.9×10^7 PFU respectively). However, other means, for example, infection with radioactively labelled virus, would be needed to exclude inefficient adsorption more rigorously.

Table 4 summarizes the results of several experiments where the reversibility of the inhibition of virus production by α -difluoromethylornithine was studied. Of the diamines studied, putrescine and cadaverine produced a complete and 1,6-diaminohexane a partial reversal of the inhibition, whereas 1,3-diaminopropane was completely ineffective. Of the naturally occurring polyamines and synthetic polyamine homologues, spermidine and *sym*-norspermine were equally effective. Diethylenetriamine and *sym*-norspermidine could not meet the polyamine requirement for virus synthesis. Thus the reversal showed considerable structural specificity, excluding the possibility that polyamines in this system acted as simple cations.

Table 2. Effect of α -difluoromethylornithine on the growth of SF virus

BHK21 cells (3.5×10^5 cells/dish) were grown for 72 h in the presence or absence of 2 mM- α -difluoromethylornithine before infection with SF virus. α -Difluoromethylornithine was also present during virus growth but not during the 1 h adsorption period. The results of two experiments are shown. The numbers in parentheses indicate virus production as a percentage of the control. Each value is the mean of duplicate assays of two separate cultures.

Time after infection (h)	Virus production in α -difluoromethylornithine-treated cultures (PFU/ μg of cellular DNA)			
	Expt. A		Expt. B	
	Control	Treated	Control	Treated
4	5.6×10^5	9.1×10^4 (16)	3.9×10^6	3.6×10^5 (9)
6	3.3×10^7	7.4×10^6 (22)	5.4×10^7	7.9×10^6 (15)
8	6.6×10^7	1.4×10^7 (21)	1.5×10^8	4.9×10^7 (34)
12	2.4×10^8	2.6×10^8 (107)	3.1×10^8	2.1×10^8 (68)
24	3.9×10^8	3.4×10^8 (88)	4.0×10^8	5.0×10^8 (125)

Table 3. *Effect of the multiplicity of infection on SF virus production in normal and α -difluoromethylornithine-treated BHK21 cells*

Cells were grown for 72 h in the presence or absence of 2 mM- α -difluoromethylornithine before infection with SF virus (5 or 50 PFU/cell). Spermidine (10 μ M) was added 24 h before infection. Virus production was measured 8 h after infection. Virus production as a percentage of control cultures is shown in parentheses. Each value is the mean of duplicate assays of two separate cultures.

Treatment		Virus production (PFU/ μ g of cellular DNA)	
α -Difluoromethyl- ornithine	Spermidine	5 PFU/cell	50 PFU/cell
—	—	1.9×10^7 (100)	2.6×10^7 (100)
+	—	3.1×10^5 (2)	1.1×10^6 (4)
+	+	3.9×10^7 (201)	2.9×10^7 (109)

Table 4. *Reversal of the inhibition of virus production by diamines and polyamines*

BHK21 cells were grown for 72 h in Eagle's minimal essential medium supplemented with 10% foetal-calf serum in the presence or absence of 2 mM- α -difluoromethylornithine before infection with SF virus. Amines (10 μ M) and iproniazid (1 mM) were added to the growth medium 24 h before infection and were also present during virus growth. Virus titre, determined 8 h after infection, is expressed as a percentage of control cultures (grown in the absence of α -difluoromethylornithine). The values are means and ranges of several experiments (the number shown in parentheses). In each experiment virus titres were determined in duplicates from two separate cultures.

Amine added	Virus production	
	Mean	Range
None (4)	6	3–8
1,3-Diaminopropane (3)	9	2–13
Putrescine (4)	90	61–143
Cadaverine (3)	91	80–107
1,6-Diaminohexane (3)	59	41–80
Diethylenetriamine (2)	6	4–9
Triethylenetetra-amine (2)	36	29–43
<i>sym</i> -Norspermidine (2)	15	12–17
Spermidine (4)	99	66–112
<i>sym</i> -Norspermine (3)	83	54–124
Spermine (2)	56	49–62

merase activity resides in the mitochondrial pellet obtained by differential centrifugation of broken cells (Ranki & Kääriäinen, 1979). The mitochondrial pellet was used in the following experiments as the source of viral RNA polymerase.

Table 5 compares virus production and viral RNA polymerase activity of BHK21 cells pretreated for 24–72 h with α -difluoromethylornithine. In this experiment the virus yield and RNA polymerase activity were determined at 5 h after infection, at which time the RNA polymerase activity has reached its plateau value (see Kääriäinen & Söderlund, 1978). As seen in Table 5, both virus production and polymerase activity decreased with increasing pretreatment time. These data suggest that inhibition of viral RNA synthesis may contribute to the low initial rate of virus production in α -difluoromethylornithine-treated cultures.

Kinetics of viral RNA synthesis *in vitro* was studied at 23°C and 37°C using the mitochondrial fraction of infected cells grown in the presence or absence of α -difluoromethylornithine as the enzyme source. Incorporation of radioactivity from [³H]-UTP into acid-insoluble material continued fairly linearly up to 15 min especially at the lower incubation temperature. There were no significant differences in the kinetics of RNA synthesis between enzyme-template preparations obtained from α -difluoromethylornithine-treated and untreated cultures (results not shown).

Inhibition of viral RNA polymerase activity by pretreatment of cells with α -difluoromethylornithine was rapidly reversed by the addition of 10 μ M-spermidine to the culture medium (Table 6). In fact, the addition of spermidine even 3 h after infection, i.e. 2 h before the isolation of the enzyme-template complex, doubled the enzyme activity *in vitro*. Neither putrescine nor spermidine had a significant effect on the enzyme activity when added directly to the assay mixture at concentrations of 0.1–1 mM (results not shown).

Effect of α -difluoromethylornithine on viral RNA polymerase

The synthesis of viral RNA in SF-virus-infected cells takes place in association with intracellular membranes, and the ability to incorporate labelled nucleotides into RNA in the presence of actinomycin D can be taken as a rough measure of RNA polymerase activity in the infected cells (Kääriäinen & Söderlund, 1978; Ranki & Kääriäinen, 1979). The template- and membrane-bound RNA poly-

Table 5. *Virus production and viral RNA polymerase activity in α -difluoromethylornithine-treated BHK21 cells*
 BHK21 cells were grown in the presence or absence of 2 mM- α -difluoromethylornithine before infection with SF virus. Virus titre was measured from the medium and enzyme-template complex isolated from the cells 5 h after infection. DNA was determined from the nuclear fraction of broken cells. Each value is the mean of duplicate assays of two pooled samples, each obtained by combining cells of two to three separate cultures. Values in parentheses are percentages of the respective control values.

Time of treatment (h)	Virus production (PFU/ μ g of DNA)		RNA polymerase activity (pmol of [3 H]UMP/15 min per mg of protein)	
	Control	α -Difluoromethylornithine	Control	α -Difluoromethylornithine
24	6.1×10^6	7.3×10^6 (119)	101.4	90.1 (89)
48	1.0×10^7	7.9×10^6 (79)	93.1	60.8 (65)
72	2.9×10^6	3.8×10^5 (13)	88.9	22.5 (25)

Table 6. *Reversal by spermidine of the inhibition of SF-virus RNA polymerase in BHK21 cells treated with α -difluoromethylornithine*

Before infection with SF virus, BHK21 cells were grown for 72 h in the presence or absence of 2 mM- α -difluoromethylornithine. Iproniazid (1 mM) was added to all cultures 24 h before infection. The concentration of spermidine was 10 μ M. Cells were harvested 5 h after infection. Each value represents the mean of two cooled samples, each obtained by combining cells of two to three separate cultures. The activities obtained from duplicate samples agreed within $\pm 6\%$.

Treatment		SF-virus RNA polymerase activity	
α -Difluoromethylornithine	Addition of spermidine	(pmol/15 min per mg of protein)	(% of control)
+	None	33	15
+	5 h after infection	37	17
+	4 h after infection	52	23
+	3 h after infection	82	37
+	1 h after infection	98	44
+	6 h before infection	110	49
+	24 h before infection	223	100
Control	None	223	100

Discussion

In the present study we have used α -difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase (Mamont *et al.*, 1978), to deplete the cellular polyamine pool. As reported previously (Raina *et al.*, 1981b), α -difluoromethylornithine at the concentration used in this study (2 mM) caused a rapid drop in putrescine and spermidine (to less than 10% and 30% of the control cultures respectively) within 24 h but had little effect on the concentration of spermine in BHK21 cells, even during a prolonged treatment. Under these conditions the net accumulation of DNA was unchanged at 24 h but was decreased by 30% at 48 h and by 60% at 72 h after addition of the drug (Raina *et al.*, 1981b). The rapid fall of cellular putrescine and spermidine is contrasted with the slow appearance of the inhibition of virus production in polyamine-depleted cells, taking place only after a pretreatment for 3 days

with the inhibitor. This observation seems to indicate that virus replication as such is not sensitive to polyamine depletion but that polyamine deficiency would slowly impair the cell machinery needed for virus synthesis. Although in principle every step of SF-virus replication from adsorption to the release of infectious virus particles may be affected, on the basis of these results it appears unlikely that inefficiency of adsorption is the main or even an important reason for the reduced SF-virus production (Table 3).

The subsequent steps of virus entry after adsorption are poorly understood (see Kääriäinen & Söderlund, 1978). Whatever the mechanism, the nucleocapsids are released into the cytoplasm and uncoated. The 42S RNA genome becomes associated with ribosomes, acting as a template for translation of non-structural proteins (which are components of the RNA polymerase). This primary translation is followed by primary transcription,

secondary translation and secondary transcription (Kääriäinen & Söderlund, 1978). It is assumed that viral RNA synthesis takes place in cytoplasmic vacuoles. In the present study we have demonstrated that the synthesis of viral RNA by the mitochondrial fraction isolated from α -difluoromethylornithine-treated cells was markedly reduced. At present it is not possible to single out the defective step(s) in the series of metabolic events after virus adsorption. Polyamine depletion may cause disturbances in the assembly and function of ribosomes, as shown by polyamine-deficient bacteria (see Goldemberg & Algranati, 1981) or in membrane function. Interestingly, it has recently been reported that polyamine starvation caused disappearance of the actin filaments and microtubules in polyamine-auxotrophic Chinese hamster ovary cells (Pohjanpelto *et al.*, 1981). It is not known whether a similar phenomenon occurs in α -difluoromethylornithine-treated cells.

The present results with SF virus together with our previous reports on HSV-2 (Tuomi *et al.*, 1980a; Raina *et al.*, 1981b) show that polyamines are needed for optimum rate of the replication of these viruses. We have recently observed that the production of Vesicular Stomatitis Virus is also markedly inhibited in α -difluoromethylornithine-treated cells (K. Tuomi, unpublished work). Some factors contributing to earlier discrepant results obtained with inhibitors of polyamine synthesis have already been mentioned (see the introduction). The present results add one more factor to the list, as it was observed (see Table 2) that the kinetics of virus production in polyamine-depleted cells differed from that of normal cells. As the rate of virus production in control cells declined earlier than in the inhibitor-treated cells, the inhibition of virus production in polyamine-depleted cells may remain unnoticed, if the virus titre is determined only at later stages of infection.

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References

- Bethell, D. R. & Pegg, A. E. (1979) *Biochem. J.* **180**, 87–94
- Cohen, S. S. & McCormick, F. P. (1979) *Adv. Virus Res.* **24**, 331–387
- Eloranta, T. O., Mäenpää, P. H. & Raina, A. M. (1976) *Biochem. J.* **154**, 95–103
- Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93
- Goldemberg, S. H. & Algranati, I. D. (1981) *Med. Biol.* **59**, 360–367
- Hafner, E. W., Tabor, C. W. & Tabor, H. (1979) *J. Biol. Chem.* **254**, 12419–12426
- Isom, H. C. & Pegg, A. E. (1979) *Biochim. Biophys. Acta* **564**, 402–413
- Jänne, J., Pösö, H. & Raina, A. (1978) *Biochim. Biophys. Acta* **473**, 241–293
- Kääriäinen, L. & Söderlund, H. (1978) *Curr. Top. Microbiol. Immunol.* **82**, 15–69
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mamont, P. S., Duchesne, M.-C., Grove, J. & Bey, P. (1978) *Biochem. Biophys. Res. Commun.* **81**, 58–66
- McCormick, F. P. & Newton, A. A. (1975) *J. Gen. Virol.* **27**, 25–33
- Pajula, R.-L., Raina, A. & Eloranta, T. (1979) *Eur. J. Biochem.* **101**, 619–626
- Palmiter, R. D. (1974) *Biochemistry* **13**, 3606–3615
- Pegg, A. E. & Williams-Ashman, H. G. (1969) *J. Biol. Chem.* **244**, 682–693
- Pohjanpelto, P., Virtanen, I. & Hölttä, E. (1981) *Nature (London)* **293**, 475–477
- Raina, A. (1963) *Acta Physiol. Scand.* **60**, Suppl. **218**, 1–81
- Raina, A. & Jänne, J. (1975) *Med. Biol.* **53**, 121–147
- Raina, A., Tuomi, K. & Mäntyjärvi, R. (1981a) *Med. Biol.* **59**, 428–432
- Raina, A., Tuomi, K. & Pajula, R.-L. (1981b) *Adv. Polyamine Res.* **3**, 163–173
- Ranki, M. & Kääriäinen, L. (1970) *Ann. Med. Exp. Biol. Fenn.* **48**, 238–245
- Ranki, M. & Kääriäinen, L. (1979) *Virology* **98**, 298–307
- Tuomi, K., Mäntyjärvi, R. & Raina, A. (1980a) *FEBS Lett.* **121**, 292–294
- Tuomi, K., Raina, A. & Mäntyjärvi, R. (1980b) *FEBS Lett.* **111**, 329–332
- Tyms, A. S. & Williamson, J. D. (1980) *J. Gen. Virol.* **48**, 183–191
- Tyms, A. S., Scamans, E. & Williamson, J. D. (1979) *Biochem. Biophys. Res. Commun.* **86**, 312–318